

Complete Genome Sequence of MIDG2331, a Genetically Tractable Serovar 8 Clinical Isolate of *Actinobacillus pleuropneumoniae*

Janine T. Bossé,^a Roy R. Chaudhuri,^{b*} Yanwen Li,^a Leon G. Leanse,^a Roberto Fernandez Crespo,^a Paul Coupland,^c Matthew T. G. Holden,^{c*} Denise M. Bazzolli,^d Duncan J. Maskell,^b Alexander W. Tucker,^b Brendan W. Wren,^e Andrew N. Rycroft,^f Paul R. Langford^a on behalf of the BRaDP1T consortium

Department of Medicine, Section of Paediatrics, Imperial College London, London, United Kingdom^a; Department of Veterinary Medicine, University of Cambridge, Cambridge, United Kingdom^b; The Wellcome Trust Sanger Institute, Cambridge, United Kingdom^c; Departamento de Microbiologia, Laboratório de Genética Molecular de Micro-organismos, Universidade Federal de Viçosa, Viçosa, Brazil^d; Faculty of Infectious & Tropical Diseases, London School of Hygiene and Tropical Medicine, London, United Kingdom^e; Department of Pathology and Pathogen Biology, The Royal Veterinary College, Hatfield, United Kingdom^f

* Present address: Roy R. Chaudhuri, Department of Molecular Biology and Biotechnology, University of Sheffield, Sheffield, United Kingdom; Matthew T. G. Holden, School of Medicine, University of St. Andrews, St. Andrews, United Kingdom.

J.T.B., R.R.C., and Y.L. contributed equally to this work.

We report here the complete annotated genome sequence of a clinical serovar 8 isolate *Actinobacillus pleuropneumoniae* MIDG2331. Unlike the serovar 8 reference strain 405, MIDG2331 is amenable to genetic manipulation via natural transformation as well as conjugation, making it ideal for studies of gene function.

Received 4 December 2015 Accepted 7 December 2015 Published 28 January 2016

Citation Bossé JT, Chaudhuri RR, Li Y, Leanse LG, Fernandez Crespo R, Coupland P, Holden MTG, Bazzolli DM, Maskell DJ, Tucker AW, Wren BW, Rycroft AN, Langford PR, on behalf of the BRaDP1T consortium. 2016. Complete genome sequence of MIDG2331, a genetically tractable serovar 8 clinical isolate of *Actinobacillus pleuropneumoniae*. *Genome Announc* 4(1):e01667-15. doi:10.1128/genomeA.01667-15.

Copyright © 2016 Bossé et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](#).

Address correspondence to Paul R. Langford, p.langford@imperial.ac.uk.

Actinobacillus pleuropneumoniae is a respiratory tract pathogen of swine that causes significant economic losses worldwide. Fifteen serovars of *A. pleuropneumoniae* vary in geographic distribution (1). Serovar 8, found in many countries around the world (1), is the predominant serovar in the United Kingdom (2) and southeastern Brazil (3). A United Kingdom clinical serovar 8 isolate, MIDG2331, was shown to be highly competent for natural transformation (4) and amenable to conjugal transfer of plasmids (5). This isolate is ideally suited for facile construction of gene deletions as well as complementation using shuttle vectors such as pMC-Express and pMK-Express (6).

The complete genome of sequence of MIDG2331 was determined using a combination of Illumina GAI and PacBio RS II platforms. Illumina sequencing yielded a total of 1,121,724 read pairs of 2×76 bp, of which 1,118,490 were retained after adapter trimming using Cutadapt version 1.8.1 (7). PacBio sequencing yielded 13,273 circular consensus sequence (ccs) reads, with a mean length of 2,861.4 bp (longest 13,273 bp), and 15,276 corrected long reads, with a mean length of 5,997.7 bp (longest 17,412 bp).

Individual assembly of the Illumina and PacBio datasets using SPAdes (8) and HGAP (9), respectively, resulted in multiple-contig assemblies (65 Illumina contigs; 2 PacBio contigs). A hybrid SPAdes assembly using both Illumina and PacBio datasets resolved into a single contig that was circularized using Circlator (10) and corrected based on the PacBio ccs reads using Quiver (9), giving a final assembly of 2,337,633 bp. Automated sequence annotation was performed using Prokka

version 1.11 (11), which predicted 2,174 putative open reading frames (ORFs), 63 tRNAs, and 6 rRNA operons. The average GC content of the genome is 41.1%, similar to other members of the *Pasteurellaceae*.

No plasmid DNA was found in MIDG2331, although plasmids have been described in other strains of this species. However, we have identified a 56-kb sequence in the genome showing a high degree of similarity to previously reported Integrative Conjugative Elements of the ICE*Hin1056* family (12, 13).

We previously characterized the reference strains of *A. pleuropneumoniae* with regard to competence for natural transformation. Serovars 1, 3, 4, 5, and 8 all showed low frequencies of transformation (10^{-8} to 10^{-9}), whereas the serovar 15 reference strain, HS143, had a transformation frequency of 10^{-4} (14). Subsequently, we identified MIDG2331 as highly competent, with a transformation frequency of 10^{-5} (4). The genome of MIDG2331 contains a full set of known competence genes (14) as well as 770 copies of the 9-bp sequence ACAAGCGGT, a DNA uptake signal sequence (USS) which is highly overrepresented in the genomes of the *Apl* subclade of the *Pasteurellaceae* (15). Although the serovar 5 L20 genome contains the same complete set of competence genes and 742 copies of the *A. pleuropneumoniae* USS (16), natural transformation is not efficient in this strain. More detailed analysis of the MIDG2331 genome, the first from a highly competent *A. pleuropneumoniae* isolate, may help identify factor(s) which contribute to transformation efficiency in this bacterium.

Nucleotide sequence accession number. The complete genome sequence of MIDG2331 was deposited in GenBank under the accession number [LN908249](#).

ACKNOWLEDGMENTS

We are grateful to Susanna Williamson of the Animal and Plant Health Agency (UK) for supplying MIDG2331.

The BRaDPIT Consortium comprises: Duncan J. Maskell, Alexander W. (Dan) Tucker, Sarah E. Peters, Lucy A. Weinert, Jinhong (Tracy) Wang, Shi-Lu Luan, Roy R. Chaudhuri (University of Cambridge; present address for R. Chaudhuri is: Department of Molecular Biology and Biotechnology, University of Sheffield, Sheffield, United Kingdom); Andrew N. Rycroft, Gareth A. Maglennon, Jessica Beddow (Royal Veterinary College); Brendan W. Wren, Jon Cuccui, Vanessa S. Terra (London School of Hygiene and Tropical Medicine); and Paul R. Langford, Janine T. Bossé, Yanwen Li (Imperial College London).

FUNDING INFORMATION

Wellcome Trust provided funding to Paul Coupland and Matthew Holden under grant number 098051. Biotechnology and Biological Sciences Research Council (BBSRC) provided funding to Janine T. Bosse, Roy R. Chaudhuri, Yanwen Li, Leon G. Leanse, Roberto Fernandez Crespo, Paul Coupland, Matthew Holden, Denise Mara Soares Bazzolli, Duncan J. Maskell, Dan Tucker, Brendan W. Wren, Andrew N. Rycroft, Paul R. Langford, and BraDPIT Consortium under grant numbers BB/G020744/1, BB/G019177/1, BB/G019274/1, and BB/G018553/1. Biotechnology and Biological Sciences Research Council (BBSRC) provided funding to Paul R. Langford under grant number BB/K021109/1. MCTI | Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) provided funding to Denise Mara Soares Bazzolli under grant number PDE 201840/2011-1.

This work was supported by a Longer and Larger (LoLa) grant from the Biotechnology and Biological Sciences Research Council (grant numbers BB/G020744/1, BB/G019177/1, BB/G019274/1, and BB/G018553/1), the UK Department for Environment, Food and Rural Affairs, and Zoetis (formerly Pfizer Animal Health) awarded to the Bacterial Respiratory Diseases of Pigs-1 Technology (BRaDPIT) consortium, a grant from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; grant number PDE 201840/2011-1) awarded to D.M.B., and a BBSRC Imperial-Brazil partnering award (BB/K021109/1) awarded to P.R.L. M.T.G.H. and P.C. were supported by the Wellcome Trust (grant number 098051). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

REFERENCES

- Gottschalk M. 2015. The challenge of detecting herds subclinically infected with *Actinobacillus pleuropneumoniae*. *Vet J* 206:30–38. <http://dx.doi.org/10.1016/j.tvjl.2015.06.016>.
- O'Neill C, Jones SCP, Bossé JT, Watson CM, Williamson SM, Rycroft AN, Kroll JS, Hartley HM, Langford PR. 2010. Prevalence of *Actinobacillus pleuropneumoniae* serovars in England and Wales. *Vet Rec* 167: 661–662. <http://dx.doi.org/10.1136/vr.c5106>.
- Rossi CC, Vicente AM, Guimarães WV, Fernandes de Araújo E, Vieira de Queiroz M, Bazzolli DMS. 2013. Face to face with *Actinobacillus pleuropneumoniae*: landscape of the distribution of clinical isolates in Southeastern Brazil. *Afr J Microbiol Res* 7:2916–2924. <http://dx.doi.org/10.5897/AJMR12.2344>.
- Bossé JT, Soares-Bazzolli DM, Li Y, Wren BW, Tucker AW, Maskell DJ, Rycroft AN, Langford PR, BRaDPIT consortium. 2014. The generation of successive unmarked mutations and chromosomal insertion of heterologous genes in *Actinobacillus pleuropneumoniae* using natural transformation. *PLoS One* 9:e111252. <http://dx.doi.org/10.1371/journal.pone.0111252>.
- Bossé JT, Li Y, Walker S, Atherton T, Fernandez Crespo R, Williamson SM, Rogers J, Chaudhuri RR, Weinert LA, Oshota O, Holden MTG, Maskell DJ, Tucker AW, Wren BW, Rycroft AN, Langford PR, BRaDPIT consortium. 2015. Identification of *dfrA14* in two distinct plasmids conferring trimethoprim resistance in *Actinobacillus pleuropneumoniae*. *J Antimicrob Chemother* 70:2217–2222. <http://dx.doi.org/10.1093/jac/dkv121>.
- Bossé JT, Durham AL, Rycroft AN, Kroll JS, Langford PR. 2009. New plasmid tools for genetic analysis of *Actinobacillus pleuropneumoniae* and other *Pasteurellaceae*. *Appl Environ Microbiol* 75:6124–6131. <http://dx.doi.org/10.1128/AEM.00809-09>.
- Martin M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet journal* 17. <http://dx.doi.org/10.14806/ej.17.1.200>.
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Pribelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 19:455–477. <http://dx.doi.org/10.1089/cmb.2012.0021>.
- Chin C, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, Clum A, Copeland A, Huddleston J, Eichler EE, Turner SW, Korlach J. 2013. Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. *Nat Methods* 10:563–569. <http://dx.doi.org/10.1038/nmeth.2474>.
- Hunt M, De Silva N, Otto TD, Parkhill J, Keane JA, Harris SR. 2015. Circlator: automated circularization of genome assemblies using long sequencing reads. *bioRxiv* 023408. <http://dx.doi.org/10.1101/023408>.
- Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 30:2068–2069. <http://dx.doi.org/10.1093/bioinformatics/btu153>.
- Mohd-Zain Z, Turner SL, Cerdeño-Tárraga AM, Lilley AK, Inzana TJ, Duncan AJ, Harding RM, Hood DW, Peto TE, Crook DW. 2004. Transferable antibiotic resistance elements in *Haemophilus influenzae* share a common evolutionary origin with a diverse family of syntenic genomic islands. *J Bacteriol* 186:8114–8122. <http://dx.doi.org/10.1128/JB.186.23.8114-8122.2004>.
- Juhas M, Crook DW, Dimopoulou ID, Lunter G, Harding RM, Ferguson DJP, Hood DW. 2007. Novel type IV secretion system involved in propagation of genomic islands. *J Bacteriol* 189:761–771. <http://dx.doi.org/10.1128/JB.01327-06>.
- Bossé JT, Sinha S, Schippers T, Kroll JS, Redfield RJ, Langford PR. 2009. Natural competence in strains of *Actinobacillus pleuropneumoniae*. *FEMS Microbiol Lett* 298:124–130. <http://dx.doi.org/10.1111/j.1574-6968.2009.01706.x>.
- Redfield RJ, Findlay WA, Bossé JT, Kroll JS, Cameron AD, Nash JH. 2006. Evolution of competence and DNA uptake specificity in the *Pasteurellaceae*. *BMC Evol Biol* 6:82. <http://dx.doi.org/10.1186/1471-2148-6-82>.
- Foote SJ, Bossé JT, Bouevitch AB, Langford PR, Young NM, Nash JHE. 2008. The complete genome sequence of *Actinobacillus pleuropneumoniae* L20 (serotype 5b). *J Bacteriol* 190:1495–1496. <http://dx.doi.org/10.1128/JB.01845-07>.